STUDIES ON YEASTS IN DAIRY PRODUCTS

I. RELATIONSHIPS OF YEASTS TO DAIRY PRODUCTS*

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The budding organisms found in milk and its various derivatives are becoming of increasing concern to investigators interested in dairy products. While the known importance of these organisms in causing certain undesirable changes, such as the formation of gas in cream and in sweetened condensed milk, accounts in part for this, their possible relationship to abnormal conditions in cream not yet definitely ascribed to any particular organism, to the deterioration of butter, and to various other undesirable changes makes their study very necessary.

The ability of yeasts to grow under conditions that must be considered unfavorable for the growth of many bacteria is one of the reasons for their importance in dairy products. Certain types of molds can also withstand severe conditions such as high acid, dearth of water, etc., in the materials on which they are growing but their air requirements limit their growth in a way that the development of certain yeasts is not limited. Moreover the growth of molds, because of their development only in rather close contact with air, is usually readily evident on observation.

A number of yeasts grow in the presence of large amounts of acid. If the temperature is suitable certain yeasts can grow rapidly throughout a can of old fermented cream in which the growth of many types of bacteria has been stopped by the acid concentration, and, as a result, abnormal flavors and odors due to yeasts are extremely common in the cream coming to the creameries to which cream is shipped for considerable distances. The acid tolerance of yeasts also enables them to grow on acid curd cheeses, such as cottage and neufchatel, on certain cheeses in which ripening occurs, such as camembert, in starters, etc.

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On some of these materials, especially acid curd cheeses and starters, definite colonies or areas of growth may be formed when the products are held at temperatures that are at all favorable for growth. The lactose-fermenting yeasts are of particular importance in acid cream because they produce gas under conditions that rather effectively limit the growth of other gas-producing organisms; they cause serious losses, not only from the influence they have on the flavor and odor of the material but also because the gas commonly forces cream from the cans. In certain types of cheese, such as Swiss, the lactose-fermenting organisms also sometimes cause serious losses by producing a swelling of the product.

Some yeasts not only tolerate considerable acid but are actually favored by it and grow better in sour milk than they do in sweet milk. With certain forms the production of objectionable odors occurs much quicker and is more pronounced in milk which contains some lactic acid, either added as such or produced by S. lactis, than in milk of the same lot without acid.

Other factors that are ordinarily considered to limit the growth of microörganisms seem to have less effect on yeasts than on many other forms. Certain yeasts grow in high concentrations of NaCl and while some types of bacteria do the same thing, these latter organisms apparently are not of importance in dairy products. The production of gas in sweetened condensed milk in which the sugar is often supposed to prevent the growth of organisms is commonly due to yeasts. Although certain cocci can grow in sweetened condensed milk, their growth is comparatively slow and is of much less importance because of their usual failure to produce significant changes.

Unfavorable conditions do not seem to result in the destruction of yeasts in the way they do many bacteria. For example in butter held for considerable periods many of the bacteria may have died out while the number of yeasts may remain comparatively high and be equal to or larger than the original count. Certain cocci of course also have the ability to resist the conditions found in butter. In laboratory cultures, yeasts are commonly found in a living condition when the medium is dried until there is only a small amount along the wall of the tube.

The large size of yeast cells in comparison to that of bacterial cells suggests that a smaller number of cells would be present in material that had been fermented by yeasts than in material fermented by bacteria. This has been found to be the case and accordingly the isolation of yeasts from material showing an abnormal condition due to these organisms requires much less dilution than if bacteria are being isolated. When bacteria are present, as they are very likely to be in dairy products, and the plates are poured so as to give the usually desired distribution of colonies with these organisms, the yeasts may be largely missed. The common procedure is to restrain the bacteria by some such method as the addition of acid and then plate heavily enough to give a satisfactory number of yeast colonies per plate. The acidity which will restrain bacteria and allow yeasts to grow is however not definite. One cubic centimeter of a 1 per cent tartaric acid solution per plate (of 8 to 10 cc. of media) is commonly employed with quite satisfactory results but it does not by any means entirely limit the growth of bacteria. As amounts of acid above this are employed there is a gradual falling off in the number of yeast colonies developing and it seems that any reasonable amount of acid—that is one which will allow a good development of yeasts—will also allow the development of certain types of bacteria. Most of the bacteria encountered in yeast isolations however are types whose colonies can readily be distinguished from yeast colonies since these in general admit of the individual cells being seen along the edge of the colony with the low power of the microscope. Occasionally colonies of large cocci may be mistaken for yeast colonies but stained preparations quickly show the error by the lack of budding cells and by the smaller size.

The yeasts common in milk and its derivatives cannot be looked upon as types peculiar to these products. Although yeasts have been found in milk drawn aseptically, in general these organisms get into milk after it leaves the udder so that there must be sources of them about the stables, milk houses, etc. The yeasts common in dairy products are accordingly to be expected in other materials which have not been studied in

any detail from the standpoint of their flora, because they are products whose value is not readily influenced by microörganisms. Since such materials are important sources of microörganisms, however, their flora must eventually be studied before a clear understanding of the yeasts of dairy products can be secured.

A number of the yeasts common in dairy products do not produce visible changes when grown in milk, and this is particularly surprising because certain of them are sometimes present in very large numbers so that growth must have taken place. Whether these organisms are of no significance from the standpoint of dairying or whether their products are of importance in the deterioration of such materials as butter, etc. can only be determined after the organisms are better understood.

Among the yeasts common in dairy products there are certain groups that seem to be clearly defined as natural groups. The lactose-fermenters for example stand out quite definitely from the other yeasts; these organisms are clearly separated from the other forms because of their gas production in milk and moreover it seems that this separation is particularly significant because when gas production occurs it is very definite, and slow or questionable fermentations are not likely to occur. Accompanying the gas production there is an alcoholic odor and, in the case of litmus milk, a reddening of litmus, both of which are uncommon changes for yeasts to produce in milk. These organisms also are almost always present in milk or cream produced under the usual conditions although during the colder weather, when their growth is slow if it occurs at all, considerable amounts of the milk or cream must often be employed for the isolation.

The yeasts producing a pink color, while frequently found in materials other than dairy products, are so common in these that they are entitled to considerable attention. These organisms apparently are never present in large numbers in ordinary milk or cream but frequently form colonies on such materials as old cream, cottage cheese, starters that are being held, etc. The pink color of these forms seems always to be definite enough so that colonies can readily be detected, whether they are on dairy products or on some plating medium. This type is further characterized by the pink color occurring as a ring or sediment in

milk tubes and by the slow changes, other than the colored areas, produced in milk.

The yeasts causing a rapid digestion of milk constitute another type that is clearly defined because outside of this type the rapid digestion of milk by yeasts is not common. Moreover the colonies produced by these organisms are quite characteristic and in general the organism can readily be picked out by the colony alone.

The yeasts that are responsible for the blowing of sweetened condensed milk constitute a definite group that is perhaps most clearly characterized by the ability to grow in strong sucrose solutions. With these types should be included other organisms that can grow in concentrated sugar solutions but which have not been isolated from spoiled sweetened condensed milk. It would be expected that these organisms might be of importance in materials other than sweetened condensed milk.

While many yeasts have been isolated from dairy products and described, these descriptions are not usually based on a study of a number of cultures from different sources that are to be considered as a single species, and accordingly the descriptions commonly do not take into account the variations that occur in the particular type. Moreover, many of the descriptions are so incomplete, particularly with reference to the action of the organism on milk, that they are of little value from the standpoint of identification. As a result, it is extremely difficult to isolate yeasts from dairy products and identify them as having been previously described and named. The confusion is being increased by certain investigators designating a yeast in published results by one of its characteristics such as "the rapidly liquefying yeast," "small white yeast," etc.

The dairy section of the Iowa Agricultural Experiment Station has been interested in the yeasts of dairy products for a number of years and has published data dealing with certain of these organisms; the forms that have been considered are, however, unrelated, and have been investigated only because of some special significance attached to them. Other types of yeasts have been studied in more or less detail and it is planned to publish data and descriptions with reference to certain of these from time to time.

RESULTS OF PRELIMINARY EXPERIMENTS UPON THE EFFECT OF SEPARATING, OR CLARIFYING, AND PASTEURIZING OF A MILK UPON THE KEEPING QUALITY OF ITS MILK POWDER.*

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In a previous report of work from these laboratories (1)[1] it has been shown that the treatment such as evaporation and homogenization, received by a milk previous to manufacture into powder produces marked changes in keeping quality. The results reported also seemed to show that increased fat content lowered the keeping quality.

The observation has been made that powdered cream possesses a keeping quality superior to that of a whole-milk powder. This appears contrary to the results reported in the publication cited.

In seeking an explanation for this, it seemed probable that the only factor wherein cream differs from whole milk is that it has gone through a centrifuging process, or has been clarified, whereas whole milks do not always get this treatment prior to the drying process. There was a probability also that the removal of slime, containing leucocytes and various enzymes, might have some effect upon the susceptibility of the fat to oxidation.

EXPERIMENTAL

A large quantity of milk with 3.8 per cent fat content was divided into 3 parts. Part 1 was given only pasteurization treatment before powdering. Parts 2 and 3 were separated and all the slime from both was returned to sample 2. No. 3 therefore was the clarified sample. Parts 2 and 3 were treated similarly to Part 1 in regard to pasteurization and were then powdered. In

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[1] Presented at the Meeting of the American Dry Milk Institute, Indianapolis, Indiana, October 3, 1925.

order to make the results comparable to the usual industrial practice, the ordinary pasteurization temperature and time were used (63°C. for thirty minutes). This procedure resulted in greater variations in the values for the different samples than might have been the case had higher temperatures been used. The samples were placed in an atmosphere of oxygen at 70°C., and the time necessary to cause the fat to absorb O_2 is its induc-

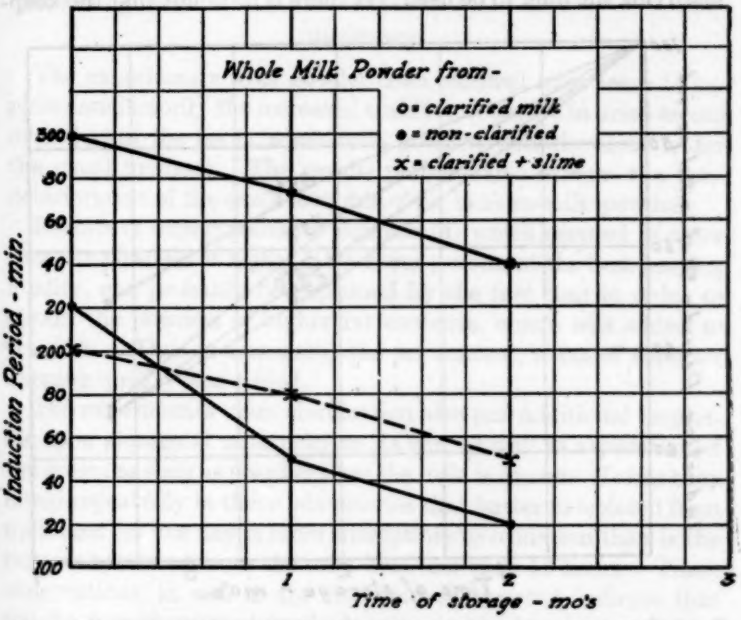

FIG. 1. SHOWING THE EFFECT OF CLARIFICATION OF A MILK UPON THE RESISTANCE OF THE FAT IN ITS POWDER TO OXIDATION

tion period for that temperature. It affords a relative method (2) for following the changes in the resistance of a fat to oxidation. Figure 1 gives the results obtained with these samples upon storage.

The check sample (nonclarified) as well as the sample which contained the separator slime deteriorated rapidly and showed

off flavor after twenty-one days' storage at room temperature. The clarified sample not only showed a good resistance to oxidation (long induction period) as soon as it had been manufactured, but at the end of two months' storage it was sweet and in good condition.

The claim is not made here that this proportionately increased keeping quality can be imparted to all milks by separating or clarifying the milk to be used; yet there is no doubt that the keeping quality can be materially improved by applying this principle to the treatment of the milk. It is evident that when higher pasteurization temperatures are used, the proportionate effect of centrifuging will become less and less, since higher temperatures tend to lessen enzymic activity.

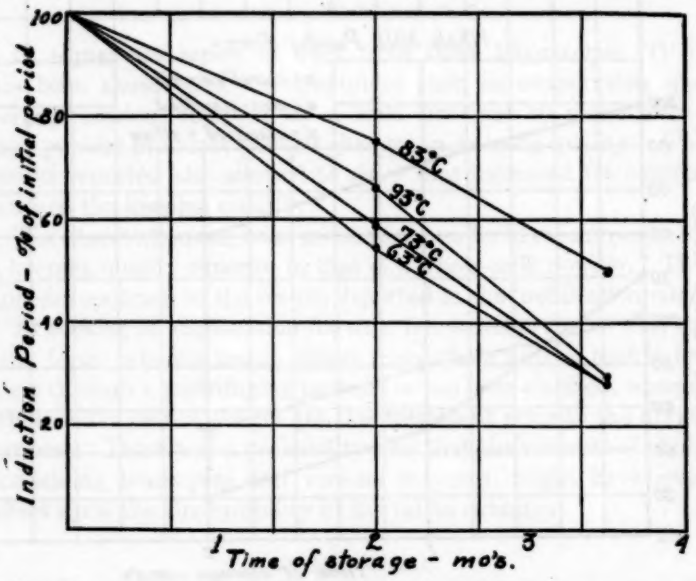

FIG. 2. SHOWING THE EFFECT OF VARIOUS TEMPERATURES OF PASTEURIZATION OF A MILK UPON THE RESISTANCE OF THE FAT IN ITS POWDER TO OXIDATION

In order to ascertain the relative quantitative effect of various

temperatures of pasteurization, four samples of whole dry milk were prepared from evaporated herd milk of 3.8 per cent fat content. The milk of sample No. 1 was pasteurized at 63°C., of sample No. 2 at 73°C., of sample No. 3 at 83°C., and of sample No. 4 at 93°C., for thirty minutes each. The samples were sealed in baby cans and stored at room temperatures, and the induction period was measured from time to time. The results are indicated in figure 2.

DISCUSSION

The experiments with powder from clarified milk seem to explain satisfactorily the increased stability of the fat in dried cream over that of the fat in whole-milk powders manufactured under the usual methods. The results perhaps also explain the nondeterioration of the small amounts of fat in skim-milk powder.

Results of experiments by Supplee (3) which seemed to show that the powders of higher fat content possessed the best keeping quality, can probably be explained by the fact that in order to obtain the powders of higher fat contents, cream was added to the milk. Thus in increasing the fat content, a fat of superior keeping quality was added.

The experiments upon clarification also put additional emphasis upon storage of milk prior to its use, as well as separation of the cream as soon as possible after the milk is drawn. It has been noted repeatedly in these laboratories that butteroil isolated from milk held for one day is more susceptible to oxidation than is the butteroil isolated from the milk held less than 12 hours. These observations, as well as the experiments reported, indicate that for the manufacture of products wherein the keeping quality of the fat is the main criterion to be considered, the time of holding should be as short as possible.

Increased temperatures of pasteurization favor increased keeping quality. A temperature of 95°C. for thirty minutes, however, seems to render the product less stable. Though this temperature is not so severe as those used in a previous work (4) to ascertain the effect of heat upon the susceptibility of fats and oils, it may well be the case that even at this temperature the detrimental effect of heat upon stability is shown.

Though preliminary in nature the results discussed, as well as numerous observations upon the keeping qualities of powders from various milks, indicate that the keeping quality of whole-milk powders can be materially improved by clarification of the milk, preferably as soon as possible after it is drawn. Higher pasteurization temperatures than are ordinarily used (63°C. for 30 minutes) also seem desirable, while too high temperatures of pasteurization (95°C.) seem to render the fat more susceptible to oxidation.

Further experiments upon an industrial scale will prove the relative value of the factors discussed to the industry.

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LACTOSE SOLUBILITY AND LACTOSE CRYSTAL FORMATION

I. LACTOSE SOLUBILITY*

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FUNDAMENTAL FACTORS IN LACTOSE SOLUBILITY

The experiments reported and results quoted in this paper on lactose solubility refer particularly to lactose solubility in the presence of sucrose and milk colloids as compared with lactose solubility in water.

In the determination of lactose solubility the chief limiting feature is the very slow rate of solution and rate of crystallization of this sugar.

This is due, as explained by Hudson (1), to the fact that lactose can exist in solution in two forms, namely as hydrated lactose and as anhydrous lactose. The stable and generally available form is the lactose hydrate. When milk sugar is placed in water in excess of the amount that is capable of going into solution, a certain portion of the sugar dissolves immediately. This represents the hydrate form of sugar in solution and is termed the initial solubility of lactose. The initial solubility is very low, amounting, according to Hudson (2) to about 8.9 parts of lactose to 100 parts of water at 25°C.

As soon as all the hydrate sugar has gone in solution that is capable of dissolving immediately, the hydrate sugar in solution begins to change to the anhydrous form, and simultaneously more of the hydrate goes into solution. This change from hydrate to anhydride and the further dissolving of hydrate continues until a point of equilibrium of the two forms in solution is reached. This equilibrium is expressed as follows:

$$\underset{\text{Hydrate}}{C_{12}H_{24}O_{12}} \rightleftharpoons \underset{\text{Beta anhydride}}{C_{12}H_{22}O_{11}} + H_2O$$

* Received for publication July 18, 1926.

and is attained when for every part of hydrate in solution, there exists about 1½ parts of anhydride in solution. When this equilibrium is established there is no further solution of milk sugar. The milk sugar solution has reached its point of saturation. This represents the final solubility. This change from hydrate to anhydride is very slow and because of this the equilibrium and final solubility are reached only after long periods of dissolving. The same principle underlies the crystallization of lactose from supersaturated solution, the rate of crystallization depending on the change of anhydride into hydrate lactose. This change also is only gradual, hence the complete crystallization of the lactose present in excess of saturation, reducing the state of supersaturation to a state of saturation, is very slow.

GENERAL PROCEDURE OF INVESTIGATION

To test out the effect of milk colloids and sucrose on the solubility of lactose various solutions were prepared in which lactose was dissolved in water, skim milk, whole milk, and in sucrose solutions of the above, respectively.

Materials used. The materials used in this investigation consisted of Merck's C. P. lactose, ranging in moisture content from 4.89 to 5.09 per cent and "Domino" brand sucrose with a moisture content of 1.5 to 2.0 per cent. Difco lactose was also used in one set of experiments, but as no material difference was noted it was decided to continue with only one brand of each sugar. All water solutions were prepared with distilled water. The whole milk used had a total solids content of 11.8 to 12.5 per cent and a fat content of 3.0 to 3.5 per cent, the total solids in the skim milk ranged from 8.69 to 10.26 per cent and the fat was 0.08 per cent or below.

In all the solutions the lactose solubility was based on the water content of the mixture rather than on actual percentage, due allowance being made for sucrose and milk solids where such were present.

To prevent the solutions from spoiling 0.5 cc. of 40 per cent formalin was added to each bottle after having determined that

the presence of the formalin in no way affected the solubility of the lactose.

EXPERIMENT 1

Lactose solubility as determined by direct observation of the saturation point

This method of determining the solubility of lactose consisted of noting the maximum amount of lactose which will dissolve in, or crystallize out from, a prearranged series of solutions of graduated concentrations.

The method used consisted of shaking a series of 8 ounce bottles sealed with well-paraffined cork stoppers previously boiled in paraffine to prevent evaporation, in a thermostatically controlled shaking chamber whereby the temperature of the bottles could be maintained constant at the temperatures used in the experiment, i.e., at 50°F. and 65°F., respectively. The lactose solubility end point was determined by noting the presence or absence of undissolved lactose or lactose crystals in the bottom of the bottles, avoiding the necessity of chemical analysis for lactose.

After a few preliminary determinations with the various solutions to be used, to roughly determine the approximate working range, it was possible to limit the number of bottles necessary to between 8 and 12 for each type of solution.

Determination A. Exactly 100 grams of water were weighed into each of 12 bottles and then after cooling to the working temperature definitely weighed amounts of lactose were added, ranging from 17.5 to 21 grams in portions differing by 0.25 gram.

These bottles were then continually shaken at constant temperature for from two weeks to one month or more. They were examined at definite intervals during this time to note the progress of solution. Complete solution required 170 hours. The experiment was, however, always continued for at least two weeks to insure complete equilibrium and final solubility.

Determination B. This was conducted in exactly the same way and with the same amount of water and lactose in each of twelve

bottles as in A but in addition 16.6 grams of sucrose were also added to each bottle. This amount of sucrose is equivalent to exactly 14 per cent, which is comparable to the sucrose content of ice cream mix and also to the approximate sucrose contained in the uncondensed milk used in manufacture of sweetened condensed milk. The sucrose in each case was completely dissolved prior to adding the lactose to avoid seeding effect.

Determination C. Still other trials of the same type as above were conducted but using as the solvent instead of water, enough whole milk and skim milk, respectively, to have present in each bottle exactly 100 grams of water. Thus for whole milk approximately 114 grams and for skim milk 110 grams were necessary.

In order to make due allowance for the lactose originally present in the milk, which was found by gravimetric analysis to be from 5.4 to 6.0 grams to each 100 grams of water in the milk, the amount of lactose added was decreased accordingly. The lactose thus added ranged from 11.5 to 15.5 grams. The calculations of the final solubility of lactose were made on the basis of the sum of the amount of lactose originally present in the milk and the amount of lactose added extraneously. This then gave results comparable to lactose solubility in pure water solutions.

Determination D. Another set of similar trials was conducted, using the same amount of whole or skim milk respectively as in C, and adding to this 18.4 grams of sucrose completely dissolved prior to the addition of the lactose. This then constituted a 14 per cent sucrose solution in milk.

Determination E. In all of the preceding trials the lactose solubility was approached from undersaturation, or from below, that is, the dry lactose was added to the cold liquid and allowed to dissolve, thus arriving at the saturation point by beginning with less than saturated solutions. In this trial the lactose solubility was determined from above in an endeavor to ascertain if the same point of solubility could be reached as with determinations from below. This was done by heating the liquid to 140°F. and completely dissolving the sucrose and lactose, then cooling to and shaking at 65°F. till equilibrium was reached. It required 5 weeks to reach a point where no more additional bottles

TABLE 1

Solubility of lactose in water, milk and 14 per cent sucrose solutions*

Results of experiment 1, trials A, B, C, D, and E

KIND OF SOLUTION	TEMPERATURE	GRAMS LACTOSE PER 100 GRAMS WATER	PER CENT LACTOSE ANHYDRIDE IN WATER	PER CENT LACTOSE ANHYDRIDE BY ANALYSIS
	°F.			
Lactose in water	50	14.97	13.0	13.02
Lactose in 14 per cent sucrose solution	50	13.93	12.4	12.63
Lactose in water	65	18.34	15.5	15.56
Lactose in water	65	18.34	15.5	15.57
Lactose in water	65	18.34	15.5	15.66
†Lactose in water crystallized from solution.	65	18.34	15.5	
Lactose in whole milk	65	18.34	15.5	15.8
Lactose in whole milk	65	18.34	15.5	
Lactose in whole milk	65	18.34	15.5	
Lactose in skim milk	65	18.34	15.5	16.1
Lactose in 14 per cent sucrose solution in water	65	17.6	14.95	14.89
Lactose in 14 per cent sucrose solution in water	65	17.6	14.95	14.94
†Lactose in 14 per cent sucrose crystallized from solution	65	18.05	15.26	
Lactose in 14 per cent whole milk with 14 per cent sucrose	65	17.4	14.84	
Lactose in 14 per cent whole milk with 14 per cent sucrose	65	17.3	14.70	15.52
Lactose in skim milk with 14 per cent sucrose.	65	17.3	14.70	15.7

* The results in above table were obtained as hydrated lactose but to facilitate comparisons they were converted into the anhydride form by multiplying by 0.95 and are expressed as such.

† Solubility determined from "above," i. e., saturation point approached from supersaturated solution as explained in trial E. All other results in this table were obtained by determining solubility from "below," i. e., approaching saturation point by allowing the lactose to dissolve to saturation.

showed lactose deposits. Twenty-four bottles were used for this test and the lactose solubility was determined in both the presence and the absence of sucrose.

The results of Determinations A, B, C, D, and E of experiment 1 are summarized in table 1.

Discussion of results in table 1. The solubility determinations in water were made chiefly for the purpose of standardizing the method of procedure, so as to make all results comparable to Hudson's determination of lactose solubility in water, whose results are shown in table 2.

From the logarithmic graph of Hudson's solubility figures[1] at different temperatures we find that the solubility of lactose at 50°F. is 13.06 per cent or 15.02 grams to 100 grams of water and at 65°F. 15.54 per cent or 18.4 grams to 100 parts of water.

TABLE 2

Final solubility of lactose by Hudson (8)

TEMPERATURE	FINAL SOLUBILITY		TEMPERATURE	FINAL SOLUBILITY	
	Lactose per cent	Parts lactose to 100 parts of water		Lactose per cent	Parts lactose to 100 parts of water
°F.			°F.		
32	10.6	11.9	120	29.8	42.4
59	14.5	16.9	147	39.7	65.8
77	17.8	21.6	165	46.3	86.2
102	24.0	31.5	192	58.2	139.2

Our results for lactose in water as given in table 1 show very close agreement with those of Hudson. At 50°F. the solubility was 13.0 per cent and at 65°F. it was 15.5 per cent. This close agreement indicates that the method used herein is correct and should give equally reliable results of lactose solubility in solutions other than water and which results may be accepted as comparable with Hudson's lactose solubility in water.

In the last column of table 1 are given additional solubility results obtained on some of the bottles containing excess lactose. In the case of water these results were obtained by total solids

[1] By plotting Hudson's figures upon a graph it will be observed that the type of curve obtained in such as to make accurate interpolation difficult. But by converting Hudson's figures into logarithms, the curve obtained becomes almost a straight line from which solubility figures for intervening temperatures may be readily derived.

determinations. These results show very close agreement with the solubility figures given in the second and third columns and thereby offer additional proof of the accuracy of this method of solubility determination. In the case of milk the chemical analyses were made by use of the gravimetric Fehling's method.

These last results on milk and sucrose milk, however, were made under conditions which may have impaired their accuracy somewhat, as some of the fat churned during the shaking and there may have been some tendency for slight inversion of the sucrose.

The results in table 1 clearly show that the lactose solubility in milk and skim milk per 100 parts of water in the milk is the same as in pure water solution. Similar results concerning the relation of colloids to lactose solubility are reported by Lucas and Spitzer (3). The sucrose in 14 per cent solution appears to have a slight solubility-diminishing effect. The per cent lactose dissolved in a 14 per cent sucrose solution was about 0.6 lower than in pure water.

EXPERIMENT 2

Lactose solubility by determination of total solids

This method of determining the solubility of lactose consisted of analyzing for total solids filtered solutions from mixtures containing lactose in excess of saturation.

After preliminary experimentation with various solutions of known lactose and lactose-sucrose concentrations it was found that the following method of drying gave reliable total solids results.

Total solids determination. Either 2 or 5 gram samples of the well mixed, filtered solutions were weighed out by the use of Mojonnier curved pipettes. Lactose solutions containing no sucrose were dried on the plain dish, since no difference was observed by drying on asbestos. Sucrose solutions and sucrose-lactose solutions were all dried on a layer of previously well dried asbestos fibre. Mojonnier solids dishes were used in all cases. The dish was first heated on the outside hot plate (180°C.) till apparently dry, then held for forty minutes in the 100°C. vacuum

oven and then for ten minute intervals till constant in weight. No further loss in weight was observed by subsequently heating at 135°C. unless when carried to the point of excessive browning.

Form of lactose in total solids determinations. Since lactose may exist in both the hydrate and the anhydrous form, there was some doubt as to which of these forms was being obtained. In order to definitely determine this, another series of preliminary determinations was made in which exactly 5 grams of Mercks lactose hydrate were dissolved in 45 grams of water; and 5 grams of sucrose were dissolved in 45 grams of water and a third solution

TABLE 3

Determination of form of lactose from solutions containing lactose, and lactose and sucrose

SAMPLE NUMBER	KIND OF SOLUTION	AMOUNT OF SUGAR ADDED	WEIGHT OF SUGAR ACTUALLY FOUND	CALCULATED WEIGHT OF ANHYDRIDE LACTOSE	DIFFERENCE
	1 gram of lactose hydrate in anhydrous form should weigh			0.9491	
1	10 per cent lactose solution	1.000	0.9437	0.9491	0.0044
2	10 per cent sucrose solution	1.000	0.9910		
3	10 per cent lactose and 10 per cent sucrose solution	2.000	1.9453		
	Lactose in solution containing 10 per cent lactose and 10 per cent sucrose	1.000	0.9543	0.9491	0.0052

was made containing 5 grams of lactose and 5 grams of sucrose in 40 grams of water. Total solids determinations were made on 10 grams of each of the above three solutions. The results of this preliminary trial are shown in table 3.

Discussion of results in table 3. The figures in table 3 show amounts of lactose approximately 5 per cent less than the amount added. This indicates that the total solids determinations obtained by the method used for solubility determinations yield the lactose in the anhydride form. The slight variations shown may well be considered within the experimental error.

It appears, therefore, correct to assume that the product

obtained in the following total solids determinations is the lactose anhydride which, as mentioned earlier, differs from the lactose hydrate form which is stable at ordinary temperatures by being 5 per cent lighter.

This fact was also mentioned by Schmöger (4) who showed that the evaporation of a lactose solution at 100°C. produced anhydrous lactose and that the dry lactose hydrate does not lose water at 100°C.

Hudson (5) also stated that the substance obtained in total solids determinations was a mixture of the alpha and beta anhydrides having the formula $C_{12}H_{22}O_{11}$. And Rice and Miscall (6) have recently demonstrated that lactose appears in the total solids residue from aqueous as well as condensed milk solutions in the anhydrous form.

Since sucrose contains a small amount of moisture, as shown under "Materials Used" earlier in this paper, due allowance was made in preparing the mixture of lactose-sucrose solutions for all lactose solubility determinations.

Total solids determinations for lactose solubility from lactose solutions in water and in 14 per cent sucrose solutions at 50°F. and 65°F. Aqueous mixtures containing lactose in slight excess of saturation and similar mixtures containing 14 per cent sucrose in addition to the lactose were continually shaken at a constant temperature of 50°F. and 65°F., respectively, for two weeks or longer. They were then filtered in an incubator maintained at the same temperature at which the mixtures had been shaken and total solids determinations were at once made on the clear filtrate. In solutions where both lactose and sucrose were present the solubility of lactose in water was obtained from the total solids figure by subtracting the known amount of sucrose previously added. The results of these determinations are assembled in table 4.

Discussion of results in table 4. The lactose solubility results obtained by the total solids determination given in table 4 show very close agreement with the results in table 1 and with Hudson's solubility figures for lactose in water. It appears, therefore, that solubility results obtained from this method of total solids deter-

TABLE 4

Solubility of lactose by total solids determinations of the filtered solutions from mixtures of lactose in water and lactose in 14 per cent sucrose solutions containing slight excess of lactose

Expressed as lactose anhydride $C_{12}H_{22}O_{11}$

SAMPLE NUMBER	KIND OF SOLUTION	TEMPERATURE	PER CENT LACTOSE ANHYDRIDE IN WATER PART OF SOLUTION	GRAMS OF LACTOSE IN 100 GRAMS WATER
		°F.		
1	100 grams of water + 16.0 grams of lactose hydrate	50	12.92 13.00	14.84 14.95
2	100 grams of water + 16.25 grams of lactose hydrate	50	13.19 12.96	15.19 14.89
3	100 grams of water + 16.5 grams of lactose hydrate	50	12.92 12.98	14.84 14.92
4	100 grams of water + 16.75 grams of lactose hydrate	50	13.03 13.05	14.98 15.00
5	100 grams of water + 17.00 grams of lactose hydrate	50	13.02 13.05	14.97 15.01
6	100 grams of water + 16.6 grams of sucrose + 15.5 grams of lactose	50	12.55 12.55	14.35 14.35
7	100 grams of water + 16.6 grams of sucrose + 15.75 grams of lactose	50	12.72 12.74	14.57 14.60
8	100 grams of water + 16.6 grams of sucrose + 16.0 grams of lactose	50	12.84 12.66	14.73 14.50
9	100 grams of water + 16.6 grams of sucrose + 16.5 grams of lactose	50	12.54 12.59 12.58	14.34 14.40 14.38
10	100 grams of water + 21.0 grams of lactose	65	15.53 15.50	18.40 18.43
11	100 grams of water + 20 grams of lactose	65	15.75 15.76	18.69 18.70
12	100 grams of water + 20.5 grams of lactose	65	15.59 15.54	18.46 18.41

TABLE 4—*Concluded*

SAMPLE NUMBER	KIND OF SOLUTION	TEMPERATURE	PER CENT LACTOSE ANHYDRIDE IN WATER PART OF SOLUTION	GRAMS OF LACTOSE IN 100 GRAMS WATER
		°F.		
13	100 grams of water + 21.0 grams of lactose	65	15.55	18.42
			15.55	18.42
14	100 grams of water + 20.0 grams of lactose	65	15.66	18.57
			15.67	18.58
15	100 grams of water + 16.6 grams of sucrose + 19.0 grams of lactose	65	14.73	17.28
			14.68	17.21
16	100 grams of water + 16.6 grams of sucrose + 19.5 grams of lactose	65	14.75	17.30
			14.80	17.37
17	100 grams of water + 16.6 grams of sucrose + 20.0 grams of lactose	65	14.53	17.00
			14.55	17.03
18	100 grams of water + 16.6 grams of sucrose + 19.0 grams of lactose	65	14.86	17.45
			14.80	17.37
19	100 grams of water + 16.6 grams of sucrose + 16.5 grams of lactose	65	14.99	17.63
			14.91	17.52
20	100 grams of water + 16.6 grams of sucrose + 17.0 grams of lactose	65	14.94	17.57
			14.85	17.44
21	100 grams of water + 16.6 grams of sucrose + 18.0 grams of lactose heated to solution and shaken for 5 weeks	65	15.03	17.69
			15.10	17.79

mination are also accurate and should yield reliable solubility figures for lactose in the presence of sucrose.

Table 4 shows that the solubility of lactose is slightly lower in 14 per cent sucrose solution than in water alone.

Lactose solubility by total solids determinations in presence of large excess of lactose. Additional determinations of lactose solubility by the total solids method were made as follows:

Solutions were prepared in 8 and 12 ounce bottles and shaken as previously described, but in this case large excesses of lactose

TABLE 5

Lactose solubility at 65°F.

Solutions prepared with large excess of lactose

SAMPLE NUMBER	KIND OF SOLUTION	TOTAL GRAMS OF LACTOSE ADDED PER 100 GRAMS OF WATER	PER CENT LACTOSE IN WATER	GRAMS LACTOSE IN SOLUTION PER 100 GRAMS WATER
1	Lactose in water	30	15.89	18.90
			15.86	18.85
2	Lactose in water	30	16.10	19.15
			16.13	19.18
3	Lactose in water	40	16.02	19.07
			16.00	19.05
4	Lactose in water	30	16.01	19.05
			15.99	19.04
5	Lactose in water	50	16.39	19.59
			16.40	19.60
6	Lactose in water	50	16.47	19.67
			16.42	19.62
7	*Lactose in water	40	16.52	19.91
			16.51	19.90
8	*Lactose in water	50	16.40	19.62
			16.38	19.59
9	*Lactose in water	60	16.66	19.99
			16.65	19.98
10	Lactose in 14 per cent sucrose solution	30	15.70	18.63
			15.68	18.61
11	*Lactose in 14 per cent sucrose solution	40	16.10	19.17
			16.00	19.09
12	Lactose in 14 per cent sucrose solution	50	15.61	18.49
			15.70	18.63
13	Lactose in 14 per cent sucrose solution	25	15.25	18.00
			15.25	18.00

TABLE 5—*Continued*

SAMPLE NUMBER	KIND OF SOLUTION	TOTAL GRAMS OF LACTOSE ADDED PER 100 GRAMS OF WATER	PER CENT LACTOSE IN WATER	GRAMS LACTOSE IN SOLUTION PER 100 GRAMS WATER
14	Lactose in 14 per cent sucrose solution	25	15.72	18.66
			15.70	18.64
15	Lactose in 62 per cent sucrose solution (163 grams sucrose)	35	13.75	15.95
			13.52	15.64
16	*Same as 62 per cent sucrose solution	35	13.59	15.68
			13.52	15.64

* Lactose solubility determined by first completely dissolving all the sugar present at 150 to 170°F. then cooling to the working temperature and allowing crystallization to proceed from the supersaturated solution until no further crystallization took place.

All results not "starred" (*) were obtained by adding the dry lactose to the cold water of the previously prepared sucrose solution and agitating until no further solution of the lactose took place.

were used. The lactose present in excess of saturation ranged from 25 to 200 per cent. After two weeks or more of shaking at constant temperature the solutions were allowed to settle and the clear liquid was poured through a filter, avoiding, as much as possible, evaporation during filtration. The mixtures used consisted of lactose in water and lactose in 14 per cent and 62 per cent sucrose solution, respectively, and total solids determinations were made as shown in table 5.

Discussion of results in table 5. The results in table 5 show slightly higher lactose solubility in solutions containing a large excess of lactose, as evidenced both from below (undersaturation) and from above (crystallization from supersaturation). This fact suggests the possible influence of the law of mass action in slightly advancing the point of equilibrium.

Similarly as in previous determinations with lesser excess of lactose, the presence of sucrose again slightly lowered the solubility of the lactose. This phenomenon becomes even more pronounced in the presence of highly concentrated sucrose solutions, as shown in the case of solutions containing 62 per cent sucrose.

Determination of lactose solubility in milk by total solids method. An attempt was made to further determine lactose solubility in milk solutions by the total solids method.

Approximately 114 grams of whole milk and 110 grams of skim milk respectively, or enough milk to give exactly 100 grams

TABLE 6

Solubility of lactose at 65°F. in milk serum after filtering off the curd

SAMPLE NUMBER	KIND OF SOLUTION	GRAMS OF LACTOSE ADDED PER 100 GRAMS WATER	PERCENT LACTOSE IN WATER	GRAMS LACTOSE TO 100 GRAMS WATER
1	Lactose in whole milk	20	15.90	18.91
2	Lactose in whole milk	25	16.45	19.69
3	Lactose in whole milk	30	16.36	19.56
4	Lactose in whole milk containing 14 per cent sucrose	20	15.69	18.62
5	Lactose in whole milk containing 14 per cent sucrose	25	15.68	18.61
6	Serum solids of whole milk soured as the above	*	5.48	
7	Lactose in skim milk	20	16.2	19.37
8	Lactose in skim milk	20	16.2	19.37
9	Lactose in skim milk	30	15.7	18.58
10	Lactose in skim milk	30	15.9	19.10
11	Lactose in skim milk containing 14 per cent sucrose	20	15.0	17.6
12	Lactose in skim milk containing 14 per cent sucrose	20	15.0	17.6
13	Lactose in skim milk containing 14 per cent sucrose	30	14.89	17.4
14	Lactose in skim milk containing 14 per cent sucrose	30	14.90	17.4
15	Serum solids of skim milk soured as in the above	*	5.87	

* No added lactose.

of water, were shaken with 20 to 30 grams of lactose powder for two weeks at 65°F. At the end of this time the casein had curdled and settled out, as no preservative had been added. By filtering off the clear serum and analyzing this for total solids an approximate estimate may be secured as to the amount of lactose in solution. Direct sugar determinations were not deemed advis-

able on these solutions due to the condition of the samples. These results are summarized in table 6.

Discussion of results in table 6. Since the milk serum separated in the manner described contains in addition to the lactose also some serum solids not lactose, amounting to about 1.5 grams per 100 grams of serum, this amount was deducted from the actual total solids figures obtained and the results are thus recorded as lactose solubility in table 6.

These figures show considerably greater variations than those previously obtained. This might be expected on account of the difficulty of preserving the original physical character of the milk during such long agitation periods.

It had been intended to determine lactose solubility by difference in total solids before and after the period of shaking but this was prevented by the churning of the fat and slight change in the casein which made filtration and centrifuging of the lactose from suspension very difficult even where preservatives were present. Representative samples of the milk as such were, therefore, considered to be out of the question and the solids determinations were made on the serum from these milk samples, which really yields the desired results, i.e., the lactose solubility in the water portion of the milk. However, the results obtained and given in table 6 may serve to show that the amount of lactose dissolved in the watery part of the milk (the serum) is practically the same as shown by results previously obtained in milk and in aqueous solutions.

EXPERIMENT 3

Lactose solubility determinations by gravimetric Fehling's method. In solutions containing very high sucrose content, such as solutions with a sucrose content of 62 per cent as is approximately found in sweetened condensed milk, and also supersaturated sucrose solutions used in this experiment, it was deemed somewhat unreliable to determine lactose by the methods previously given in this paper. The content of both sugars was, therefore, determined by the regular gravimetric Fehling's solution method using Munson and Walker's tables.

These solutions were prepared by completely dissolving the two sugars in a water bath at 150° to 170°F. and, after cooling, maintaining a temperature of 65°F. for six weeks with occasional shaking. Total solids determinations were made at intervals on the supersaturated liquid and when no further change could be detected the lactose crystals in suspension were removed by centrifuging until no crystals could be detected under the microscope. The top portion of the liquid was then stirred carefully and siphoned off as completely as possible into a dry bottle. In the case of the aqueous solutions no difficulty was experienced but in the case of the milk solutions it was necessary to repeat the centrifuging as some lactose crystals still appeared in suspension after the first separation.

The solutions prepared were as follows:

1. Sixty-two per cent sucrose-water solution containing 100 grams of water, 163 grams of sucrose and 35 grams of lactose.
2. Sixty-two per cent sucrose-milk solution containing 113.6 grams of whole milk, 163 grams of sucrose and 35 grams of lactose.
3. Supersaturated sucrose-water solution containing 100 grams of water, 230 grams of sucrose and 35 grams of lactose.
4. Supersaturated sucrose-milk solution containing 113.6 grams of whole milk, 230 grams of sucrose and 35 grams of lactose.

After cooling, the water lost by evaporation during the heating was replaced. The water of replacement contained 0.5 cc. of formaldehyde so as to check fermentation.

Preliminary lactose and lactose-sucrose determinations using known solutions of Difco lactose and Pfanstiehl chemically pure sucrose in water and in milk, of about the same sugar ratio in water as those of the experiment proper showed that no factor was necessary in determining sucrose in such solutions even in the presence of lactose, but that in the case of determining lactose in the presence of sucrose the 1 lactose-12 sucrose column of table 7 A. O. A. C. Methods (7) should be used, and that in addition the results must be multiplied by the factor 1.0173 and also by 0.95 to express the results as anhydrous lactose. All results were obtained in triplicate. The results are assembled in table 7.

Discussion of results in table 7. Since the solubility of lactose in 62 per cent sucrose-water solution above given agrees well with that obtained from total solids determinations, see table 5, this serves as an additional check on lactose solubility by the total solids determination.

These results further show that sucrose in high concentrations such as solutions containing about 62 per cent sucrose, does affect the solubility of lactose in water to a considerable extent reducing it approximately 15 per cent. Thus, as shown in table 1, 100 grams of pure water at 65°F. will dissolve approximately 18.4 grams of lactose, while 100 grams of water of a 62 per cent sucrose solution will dissolve only about 15.35 grams lactose.

TABLE 7

Solubility of lactose in 62 per cent and supersaturated sucrose water and whole milk solutions at 65°F.

Determined by Gravimetric Fehling's Method. Expressed as anhydrous lactose ($C_{12}H_{22}O_{11}$).

KIND OF SOLUTION	TOTAL SOLIDS OF WHOLE	PERCENT SUCROSE IN WATER OF SOLUTION	PERCENT LACTOSE IN WATER OF SOLUTION	GRAMS LACTOSE TO 100 GRAMS OF WATER
	per cent			
Water containing 62 per cent sucrose....	63.65	61.40	13.20	15.20
Whole milk containing 62 per cent sucrose	63.93	61.57	13.45	15.50
Supersaturated sucrose-water solution...	68.37	66.67	14.49	16.94
Supersaturated sucrose-milk solution....	69.10	66.91	14.01	16.30

This is significant in connection with sweetened condensed milk, since sweetened condensed milk which contains from 40 to 45 per cent sucrose usually has a sucrose-in-water concentration of from about 60 to 64 per cent. Sweetened condensed milk contains from about 11 to 15 per cent total lactose and from about 24 to 28 per cent water. On the basis of these figures the lactose-water ratio would range from about 28 to 38 per cent while the lactose solubility in this product is only about 13 to 14 per cent or from about 15 to 16 grams to 100 grams of water. It appears evident, therefore, that from about one-half to two-thirds of the lactose contained in sweetened condensed milk is present in excess of saturation, i.e., in crystalline form. The figures in table 7 are

of further interest in that the information they reveal is helpful in the determination of the amount of lactose crystals present in sweetened condensed milk of known composition.

The results in table 7 further show that the milk colloids in sweetened condensed milk have no noticeable effect on lactose solubility in the water portion of the milk. The amount of lactose dissolved in the sucrose milk solution being probably the same as that dissolved in the sucrose water solution, i.e., 13.2 and 13.45 per cent, respectively.

Commercial application of these facts in the manufacture of sweetened condensed milk and the use of such knowledge in controlling the smoothness of the finished product are discussed in detail by Hunziker (8).

Similarly as in the case of 62 per cent sucrose solutions, so did solutions in which the sucrose as well as the lactose was present to the extent of supersaturation, have a solubility-diminishing effect on the lactose. And again, in such solutions in milk the milk colloids appeared not to affect the lactose solubility. However, the lactose solubility results in these sucrose-supersaturated solutions, where both sugars are crystallizing do not show quite as close agreement as those from solutions of lesser sucrose concentration. It is possible that in these concentrated solutions complete crystallization may not have been definitely obtained, although the solutions were allowed to crystallize for two months.

SUMMARY

1. Lactose solubility in water has been rechecked and very close agreement was obtained with the results of Hudson.

2. Lactose solubility determinations in sucrose solutions and in milk with and without sucrose were made. The results of these determinations show that the solubility of lactose in milk was similar to that in aqueous solutions, but that the presence of sucrose decreased the lactose solubility somewhat. This decrease in lactose solubility ranged from about one-twentieth to one-sixth of the lactose solubility found in pure water. It was only slight in dilute sucrose solutions, such as solutions containing 14

per cent sucrose, and it became greater with an increase in the sucrose concentration.

3. Lactose solubility was approached both from below and

TABLE 8

*Lactose solubility averages**

KIND OF SOLUTION	TEMPERATURE	AVERAGE OF BEST RESULTS		AVERAGE OF ALL FIGURES	
		Percent lactose in water part of solution	Grams lactose to 100 grams of water in the solution	Percent lactose in water part of solution	Grams lactose to 100 grams of water in the solution
	°F				
Lactose in water	50	13.02	14.97	13.02	14.97
Lactose in water	65	15.50	18.34	15.97	19.01
Lactose in whole milk	65	15.50	18.34	15.87	18.86
Lactose in skim milk	65	15.50	18.34	15.90	18.95
Lactose in water containing 14 per cent sucrose	50	12.63	13.93	12.63	13.93
Lactose in water containing 14 per cent sucrose	65	14.95	17.60	15.16	17.88
Lactose in whole milk containing 14 per cent sucrose	65	14.77	17.30	15.25	17.98
Lactose in skim milk containing 14 per cent sucrose	65	14.70	17.30	14.89	17.46
Lactose in water containing 62 per cent sucrose	65	13.20	15.20	13.46	15.55
Lactose in milk containing 62 per cent sucrose	65	13.45	15.50	13.45	15.50
Lactose in supersaturated sucrose-water solution	65	14.49	16.94		
Lactose in supersaturated sucrose-milk solution	65	14.01	16.30		
Lactose in water (by Hudson)	50	13.06	15.02		
Lactose in water (by Hudson)	65	15.54	18.40		

* The results given in the second and third columns of the table are considered somewhat more accurate and were obtained after the method of procedure had become well established. However, the averages of all results as given in columns four and five of the table are not seriously at variance with former figures and with those by Hudson.

from above, i.e., from undersaturation by adding the dry lactose to the cold liquid and allowing it to dissolve, to saturation, and from supersaturation by dissolving the dry lactose in the heated

liquid, cooling and allowing to crystallize until equilibrium was reached. The results of the two procedures checked closely, indicating that the point of equilibrium had actually been reached.

4. Lactose solubility was determined by: first, noting the maximum amount of lactose which will dissolve in or crystallize out from prearranged series of solutions of graduated concentrations; second, total solids determinations of the filtered solutions; and third, the gravimetric Fehling's solution method. The results obtained by the different methods were in close agreement.

5. Averages of all determinations of lactose solubility for each type of solution used are given in table 8.

CONCLUSIONS

The results on lactose solubility suggest the following deductions:

1. That the colloids in milk do not have any material influence on the solubility of lactose.

2. That the presence of sucrose diminishes the solubility of lactose somewhat. This decrease is only slight in dilute sucrose solutions such as are comparable to ice cream mix, but becomes greater as the concentration of sucrose increases, the decrease in sucrose solutions, comparable to sweetened condensed milk, amounting to approximately 15 per cent of the lactose solubility in aqueous solutions.

3. It is of interest to point out here, that the presence in lactose solutions of high sucrose concentration also has a very noticeable influence on lactose crystal formation, the shape of the lactose crystals being distinctly modified. The lactose crystals from such solutions are short, blunt and apparently do not assume the typical form of lactose crystals from supersaturated solutions of lactose in water, as described and illustrated in Part II on "Lactose crystal formation" which will appear in the next issue of this JOURNAL.

Our present knowledge of this subject is too limited to justify any discussion as to the possible relation between the effect of

sucrose on lactose solubility and on the form of lactose crystals. However, these observations are suggestive of the possibility that such a relation may exist.

A similar parallel is observed between the effect of milk colloids on lactose solubility and on the form of lactose crystals, in that the milk colloids appear to have no effect in either case, as also shown in Part II on "Lactose crystal formation."

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ABSTRACTS OF FOREIGN DAIRY LITERATURE

J. L. HILEMAN

BLANCHETIERE. *Seasonal Variations of Some Mineral Elements of Milk.* (C. R. des seances de la Soc. de Biol. (France), Vol. 92, p. 1295, 1925. Abstracted in Le Lait, Vol. 6, April, 1926, p. 290.)

In mixed milk furnished to the hospitals of Lens, the alkali metals sodium and potassium diminish in amount in winter, while the alkaline earth metal calcium increases in winter. This cannot be explained by any variation in the content of these elements in the food, nor by the theory of Taylor and Usband that there is an inverse relation between the composition of the milk and the rapidity of secretion, since the different elements do not vary in the same direction.

The author explains the phenomena by the fact that the vitamin A, which is ingested in greater quantity in summer, causes a greater retention of calcium in the organism, as shown, among others, by Hart, Steenbock and Hoppert, thus leaving less available for the milk.

F. RICHARD. *Analysis of Milk Powder.* (Le Lait, Vol. 6, February, 1926, p. 86.)

In a series of comparisons, the Röse-Gottlieb method for fat in milk powder gave consistently lower results (from 0.60 to 1.90 per cent difference) than the Weibull method. (See abstract of article by Schoonjans.)

Moisture is determined in milk powder by heating five grams of powder in a tarred shallow dish at 100°C., cooling in a desiccator, weighing, and taking the loss in weight as moisture.

ALBERT SCHOONJANS. *Note on the Determination of Fat in Milk Powders.* (Le Lait, Vol. 5, October, 1925, p. 782.)

Schoonjans describes and compares results with three methods of determining fat in milk powder; the common Röse-Gottlieb, the Weibull and the Teichert.

In the Weibull method, 2.5 grams of powder are boiled in a flask for fifteen minutes with 30 cc. of eighth-normal hydrochloric acid and 20 cc. of water, adding a small amount of pumice stone to avoid bumping. The solution is then filtered while hot through a moist

paper and the residue on the filter paper is washed till free from acid. The filter paper and its contents are dried at 100°C. and extracted in a Soxhlet apparatus to determine the amount of fat. This method gives consistently higher results than the Röse-Gottlieb method, the difference between the two varying from 0.15 to 1.30 per cent.

The Teichert method is a modification of the Gerber method. Place 2.5 grams of powder, 10 cc. of sulphuric acid (specific gravity 1.81) and 1 cc. of amyl alcohol in a tube made especially for the purpose, shake, centrifuge and read the percentage of fat as in the Gerber method. This method gives results only slightly higher (from 0.05 to 0.15 per cent) than the Weibull method.

TSCHIRCH AND BARBEN. *Rancidity of Fats.* (Schweiz Apothekerztg., Vol. 62, p. 281, 1924. Abstracted in Le Lait, Vol. 6, February, 1926, p. 119.)

In order for a fat to become rancid, there must be present water, air, light, and an unsaturated fatty acid. Exposed to air and moisture, unsaturated molecule adds oxygen at each double bond, forming a peroxide. Water eliminates a part of that oxygen with the formation of an oxide, together with hydrogen peroxide and ozone. The ozone gives rise to the formation of less stable ozonides, which are split by the water into simpler molecules of odorous aldehydes, ketones and acids.

By imitating the above conditions with lead peroxide (PbO_2) and sulphuric acid, the author produced rancidity in peanut oil. In the rancid fat, one can easily detect hydrogen peroxide: it gives the Kreis reaction.

This author apparently uses the term rancid in the sense in which we use tallowy, since the fat was described as having the odor of tallow.

WEIGMANN AND TODT. *Experiments on the Manufacture of Cheese with Pasteurized Milk.* (Molkerei Zeitung (Hildesheim), Nos. 46, 47, and 48, June, 1923. Abstracted in Le Lait, Vol. 6, February, 1926, p. 130).

Milk was heated at 110°C. and made into cheese after adding calcium chloride and a lactic starter. The rennet acted normally and the curd was malleable under pressure, but the cheese did not ripen properly and was worthless.

Satisfactory cheese can be made from milk heated to from 95° to

97°C., and also from milk held for thirty minutes at 63°C. The former is less open in texture.

Pasteurization retards ripening, and the cheese from pasteurized milk has a smoother rind and is less subject to mold growth than cheese from unpasteurized milk.

L. LINDET. *On the Coagulation of Casein in the Presence of Salts of Lime in Acid Solution.* (Le Lait, Vol. 5, December, 1925, p. 953.)

Casein precipitated from skim milk by lactic acid or other acids and treated with an excess of acid forms a jelly-like solution. The casein can be reprecipitated from this solution by the addition of calcium salts. The reprecipitated casein contains approximately the same amount of phosphoric acid as the original precipitate (about 2 per cent P_2O_5) but almost no lime, whereas the original precipitate contained from 0.89 to 1.5 per cent CaO.

The author draws the conclusion that acid alone is not capable of bringing about the coagulation of casein, but that the presence of calcium salts in solution is necessary. The data presented in support of this conclusion are rather meager.

W. DORNER. *A Simple Procedure for the Staining of Spores.* (Le Lait, Vol. 6, January, 1926, p. 8.)

This method is a combination of the negative staining method of Burri, in which the bacteria are not stained but are surrounded by a black background of India ink or nigrosine B, and of Klein's method of staining spores. The nigrosine B takes the place of alcohol in decolorizing the bacterial cells after staining with carbol fuchsin, leaving the spores a bright red, the bacterial cells surrounding the spore colorless, and the background black.

The technique of the method is as follows:

1. Prepare a suspension of the bacteria to be stained in 0.5 cc. of water in a centrifuge tube.
2. Add 1 to 2 cc. of a melted 10 per cent gelatin solution.
3. Add 1 to 2 cc. of carbol fuchsin solution.
4. Heat the tube in a boiling water-bath for ten minutes.
5. Wash the bacteria to remove the gelatin. To do this, add 10 to 15 cc. of water, and centrifuge for some minutes, or allow to settle for several hours.
6. Mix a loop of the sediment from the bottom of the tube with a loop of a saturated aqueous solution of nigrosine B on a slide and dry.
7. Examine with an oil-immersion lens.

BLEYER AND KALLMAN. *Contribution to the Knowledge of Some Little-Studied Components of Milk.* (Biochemische Zeitschrift, 153, Nos. 3–6, pp. 459. Abstracted in Le Lait, Vol. 6, January, 1926, p. 26.)

These authors find that the nitrogenous bodies in milk are casein, albumin (globulin and pseudoglobulin), albumose, peptone, and small amounts of purines, uric acid, urea, ammonia, creatine, creatinine, amino acids, sulphocyanide, and a nitrogenous coloring matter.

HAGLUND, BARTHEL AND SANDBERG. *The Number of Bacteria in Milk for Cheese-Making and the Rapidity of Ripening of the Cheese.* (Communication No. 270, Central Agricultural Experiment Station. Bacteriological Section No. 35, Stockholm, 1924. Abstracted in Le Lait, Vol. 6, January, 1926, p. 31.)

Increasing either the number of bacteria or the acidity of the milk independently at the time of coagulation increases the rapidity of the ripening of the cheese, as measured by the quantity of soluble nitrogen compounds formed during a certain period of time.

DAIRY NOTES

INTERNATIONAL DAIRY CONGRESS

The following information in regard to the plans to hold an International Dairy Congress in England in 1928, has been taken from recent numbers of "The Milk Industry" published in London.

Preparations for the Congress are proceeding apace. Meetings of the General Committee were held in April and in June. Mr. R. Ravenscroft, Secretary of the British Dairy Farmers' Association, 28 Russell Square, London, S. W. 1, England, is acting as Secretary for the Congress and is working with the greatest enthusiasm to make the Congress a success. A provisional committee has worked out a tentative program with estimates of the cost and has satisfied the Executive Committee as to the practicability of holding the Congress.

It is proposed that the Congress should cover a period of ten days, three of which would be spent in London, two in Reading, two at the Royal Agricultural Society's Show to be held at Warwick and the remaining three days to be spent in Scotland. In order to include the Royal Show it will be necessary to hold the Congress during the first week of July. Lord Kenyon has accepted the Presidency and extensive preparations are being made for the entertainment of visiting delegates.

Among the subjects recommended for papers are the breeding and feeding of animals for milk production, milk transportation, milk distribution, organization for advisory assistance, dairy research, dairy machinery and equipment, international questions and coöperation.

The placing of the Congress during the early part of the summer will make it necessary for those who go directly from North America to the Congress to travel at the height of the tourist season. For this reason plans must be worked out a long time in advance. Preliminary inquiries are already being made to determine the number of Americans who are likely to go, the thought being that a group might like to join in securing space on the same steamer.

R. S. BREED.

CONNECTICUT. Prof. George C. White, head of the Dairy Department and Dean of the College of Agriculture, has been at Ithaca, N. Y.,

since September 1. His leave of absence carries through to the first of February, 1927. He is pursuing special studies in animal diseases and in the field of education at Cornell University. Professor White is secretary of the American Dairy Science Association and he may be addressed care of the Department of Dairy Industry at Cornell University.

MASSACHUSETTS. Mr. Richard Smith, Jr., has returned to the dairy staff after a year's leave of absence during which time he did graduate study in the dairy husbandry department of the University of Illinois.

NEW YORK. Mr. J. C. Marquardt of the Experiment Station at Geneva, N. Y. has recently returned from Cornell University where he has completed the requirements for the degree of Master of Science. Mr. J. C. Hening will study at the same institution for an advanced degree this college year.

NEW YORK, CORNELL COLLEGE. Prof. H. E. Ross has recently returned from his sabbatic leave of absence of one year, which he spent in Argentina aiding the Government in the improvement of the city milk supply and in the installation of modified milk laboratories.

Prof. J. D. Brew is on sabbatic leave for one year. He is doing graduate work at the University of California.

Prof. W. W. Fisk is on a leave of absence for one and one-half years during which time he will be engaged in commercial activities.

Dr. Arthur T. Henrici, Professor of Bacteriology and Immunology of the Medical College of the University of Minnesota, has been appointed acting Professor for the year 1926–27. During this period Dr. Henrici will be on sabbatic leave from the University of Minnesota.

Dr. Phil. Karl Demeter, head of the Bacteriological Department in the South German Research Institute for Dairy Science, Weihenstephan, near Munich, Germany, will sail for home on October 14. Dr. Demeter has been doing special research in the department of dairy industry, and is one of many young scientists who have had opportunity to come to America under the auspices of the International Education Board.

Dr. Norman C. Wright, assistant research chemist at the National Institute for Research in Dairying at the University of Reading, Reading, England, has just arrived at Cornell University for two years of research study in the department of dairy industry. Dr. Wright is a

fellow on the Commonwealth Fund. He will have opportunity during his two years of study in America to travel three months each year.

Other recent appointments include Georges Knaysi, Instructor in Bacteriology; Walter Hochstrasser, Instructor in Dairy Industry, and G. M. Bateman, Instructor in Dairy Chemistry.

ERRATUM

In the September number of the JOURNAL OF DAIRY SCIENCE the chart on page 486 should be on page 485 with the legend—"Fig. 1. Effect of heating at 101.5°C. on the hydrogen-ion concentration and on the titratable acidity of milk." The chart on page 485 should be on page 486 with the legend—"Fig. 2. Effect of heating at 95°C. on the hydrogen-ion concentration and on the titratable acidity of milk."

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